

## REMARKS

Claims 1–13, 15–19, 21, 23, and 24 are pending in the application. Claims 1–13, 15–19, 21, 23, and 24 stand rejected. Claims 1 and 24 have been amended. Claims 15 and 16 have been canceled. Reconsideration and allowance of Claims 1–13, 17–19, 21, 23, and 24 is respectfully requested.

The Rejection of Claims 1-13, 15–19, 21, 23, and 24 Under 35 U.S.C. § 102(B) as Being Anticipated by U.S. Patent No. 5,294,549 (Pullman et al.)

Claims 1–13, 15–19, 21, 23, and 24 stand rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 5,294,549 (Pullman et al.). Applicants traverse this ground of rejection for at least the following reasons.

While not acquiescing to the Examiner's position, but in order to facilitate prosecution, Claim 1 has been amended at step (b) to recite

cultivating pre-cotyledonary pine embryogenic cells from step (a) for a period from one week to two weeks in, or on, a synchronization medium that comprises an absorbent composition and at least one synchronization agent selected from the group consisting of abscisic acid and a gibberellin, wherein the absorbent composition and the at least one synchronization agent are present at a concentration effective to produce a synchronized population of pre-cotyledonary pine somatic embryos wherein at least 50% of the pre-cotyledonary pine somatic embryos in the synchronized population are at the same developmental stage . . . .

Support for this amendment is found in the specification as filed, for example at page 6, lines 25–28; page 16, lines 26–30; and page 18, lines 8–11.

Claims 1 and 24 have further been amended to remove the phrase "wherein the osmolality of the maintenance medium is from 180 mM/Kg to 400 mM/Kg." No new matter has been introduced.

#### Overview of the Invention

The claimed invention is based on the discovery by the present inventors that culturing pine embryos in a synchronization medium containing an absorbent composition (e.g., activated charcoal) and at least one of abscisic acid and a gibberellin for one to two weeks prior to incubation in development media inhibited precocious embryo development and greening, while promoting synchronization of the cultures, resulting in embryos very uniform in size in comparison to control cultures. See specification at page 19, lines 19–31.

#### The Pullman et al. Reference

The Examiner characterizes Pullman et al. as disclosing a method of cultivating conifer pre-cotyledonary somatic embryos in a maintenance medium comprising nutrients that sustain the embryos, having an osmolality of 170 mM/Kg to about 240 mM/Kg (Col. 15, lines 1–3), followed by transfer to a singulation medium comprising gibberellin and/or abscisic acid at a concentration of 0.05 and 15 mg/L (Col. 13, lines 40–60) and activated charcoal (Col. 13, lines 50–54) for at least three weeks (Col. 15, lines 23–26), and finally, transfer to a development medium wherein the osmolality is about 400 mM/Kg (Col. 15, line 60).

As acknowledged by the Examiner, Pullman et al. does not disclose or suggest cultivating pre-cotyledonary pine embryogenic cells for a period from one week to two weeks in or on a synchronization medium, as claimed. In order to anticipate, the reference must disclose, either expressly or inherently, each and every element of the claim. M.P.E.P. § 2131. Therefore, the claimed invention, as amended, is not anticipated by Pullman et al.

Moreover, Pullman et al. does not suggest or provide any motivation to carry out the claimed method as amended, which comprises cultivating pre-cotyledonary pine embryogenic cells for a period from one week to two weeks in or on a synchronization medium for at least the following reasons.

Pullman et al. discloses an intermediate culturing step referred to as "singulation" for Douglas-fir, which Pullman states is "not necessary for other species." See Pullman et al. at Col. 8, lines 18-21. Pullman et al. teaches the transfer of pre-cotyledonary Douglas-fir somatic embryos from a maintenance medium to a singulation medium for at least three weeks, followed by transfer to a development medium. As described in Examples 1-7, which are directed to methods for improving Douglas-fir embryo development, "Late stage Douglas-fir proembryos were singulated in a three step liquid shake culture as outlined above." Example 2 at Col. 15, line 68, to Col. 16, line 2. As described in Example 1, a preferred schedule for the singulation step in Douglas-fir is "one week on a medium containing 10mg/L ABA, a second week on a medium containing 5/mg/L ABA, and a third week on a medium also with 5mg/L ABA." Col. 15, lines 10-27.

It is further noted that in Examples 8 and 9 of Pullman et al., which are directed to methods for improving *Norway Spruce* embryo development, no singulation step was carried out, which is consistent with the statement made earlier in Pullman et al. that the singulation is required for Douglas-fir but is "not necessary for other species." See Pullman et al. at Col. 8, lines 18-21. As further described in Examples 8 and 9, Norway Spruce late stage proembryos were plated directly from a maintenance medium onto solid development media containing various concentrations of ABA and GA.

Therefore, it is demonstrated that there is no teaching or motivation provided in Pullman et al. that would lead one to culture pre-cotyledonary pine embryos in a synchronization medium for a period from one week to two weeks, as claimed.

Further, the invention of Claim 1 cannot be considered to be obvious over Pullman et al. because there is no teaching or suggestion in Pullman et al. to modify the teachings of Pullman et al. to arrive at the claimed invention. Importantly, Pullman et al. does not remotely teach, suggest, or provide any motivation to produce a synchronized population of pine somatic embryos, as claimed. As described in the instant specification, "Cleavage polyembryony (embryonal suspensor mass proliferation) continues in cultures after plating onto development medium, and new embryos are beginning to develop even after eight to ten weeks of culture on development medium. Due to this continuing cleavage, embryos are not uniform in stage, shape, size or quality within a single plate." Specification at page 4, lines 18–20.

As described in Examples 1 and 2 of the instant specification, the present inventors determined through experimentation that a synchronized population of mature pine somatic embryos could be obtained by culturing pre-cotyledonary pine embryogenic cells in a synchronization media containing activated charcoal and at least one of abscisic acid and a gibberellin for one to two weeks, followed by incubation in a development media inhibited precocious embryo development and greening, while promoting synchronization of the cultures, resulting in embryos very uniform in size in comparison to control cultures. See specification at page 19, lines 19–31. As further described in Example 2 of the instant specification, it was experimentally demonstrated that in the absence of the step of culturing in a synchronization medium (i.e., control cultures grown in maintenance medium and directly transferred to development media, similar to Examples 8 and 9 in Pullman et al.), the cultures contained

embryos that were cleaving, growing and forming embryo suspensor masses, with embryos seen in many different stages. Specification at page 19, lines 1-5.

Because Pullman et al. does not disclose or suggest culturing pine embryos in a synchronization medium for one to two weeks prior to development, as claimed, the cited reference fails to teach or suggest all the elements of the claimed invention, and therefore does not anticipate or render obvious the method of the claimed invention. Thus, without the benefit of the applicants' disclosure, one of skill in the art would not be motivated by the teachings of the cited reference or by the general knowledge in the art to arrive at the claimed invention, and would have no reasonable expectation of success in practicing the invention as claimed.

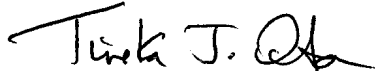
Accordingly, because Pullman et al. does not disclose every element of Claim 1 and because the general knowledge of one skilled in the art would not provide any basis or motivation to arrive at the claimed invention, Claims 1-13, 17-19, 21, 23, and 24 are believed to be clearly patentable under both 35 U.S.C. §§ 102 and 103 over Pullman et al.

#### CONCLUSION

In view of the foregoing, applicants submit that all of the pending claims are in condition for allowance and notification to this effect is respectfully requested. The Examiner is further requested to contact the applicants' representative at the number set forth below to discuss any issues that may facilitate prosecution of this application.

Respectfully submitted,

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